while arterial pressure increased. Venous pressure increased while forearm venous distensibility and venous volume decreased in each subject. In each case blood shifted from the forearm. These experiments indicate that mephentermine administration increases both peripheral resistance and venous tone in man.

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A Species Comparison of Serum Proteins and Enzymes by Starch Gel Electrophoresis.* (26180)

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Employment of starch gel electrophoresis as a means of protein separation has found wide application. Serum proteins(1), hormones(2), serum(3,4) and tissue(5) enzymes have been characterized by this technic. Although several recent investigations have been concerned with the enzymes of human serum(3,4), little information is available on the enzymes of laboratory animals. Protein migration in a number of animals(6-11) and esterase migration in starch block in several species(12) have been published. In the present study the occurrence and distribution of esterase, cholinesterase, aminopeptidase, cytochrome oxidase, beta-glucuronidase, acid and alkaline phosphatase and ceruloplasmin oxidase in the sera of man and 6 species of experimental animals are compared. Succinic, lactic, beta-hydroxybutyric and glutamic dehydrogenases were also studied in human serum. To provide a broader basis of comparison and for orientation, serum protein patterns are also included.

Materials and methods. Species studied and number of individuals in each follow: Man (Caucasian) 30, monkey (Cynomologus from Philippines) 3, dog (mongrel) 3, rabbit (New Zealand White) 8, guinea pig (hybrid) 8, rat (Sprague-Dawley) 8, mouse (Webster strain of Swiss) 10. All subjects were adult males.

Starch gel electrophoresis was carried out under conditions described by Smithies(1). Specimens were absorbed on filter paper and inserted in a slot 10 cm from the cathode in parallel with a specimen of human serum. Following electrophoresis, the gels were sliced in half longitudinally. One half was stained for protein with Amido-black 10 B and enzymatic activity was determined in the other (4). Gels were photographed and schematic representations prepared. Differences in mi-

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gration were compensated by differential enlargement employing the leading edge of the human albumin band as an index.

Technic of Stains: *Aminopeptidase.* 50 ml Tris-maleate buffer, 0.1 M, pH 6.8; 20 mg L-leucyl-beta-naphthylamide (Dejac Laboratories; this dissolves with difficulty but should not be heated); 30 mg Black K salt; incubate 3 hours at 37°C with occasional gentle shaking.

Beta-glucuronidase. 30 ml acetate buffer, 0.1 M, pH 5.2; 15 mg 8-hydroxy-quinoline glucuronide (dissolves with difficulty but can be safely heated to the temperature of the paraffin oven, 56°C); 20 mg Blue RR salt; incubate 16 hours at 37°C.

Cholinesterase. 50 ml Tris-maleate buffer, 0.1 M, pH 6.4; 20 mg 6-bromo-2-naphthyl-carbonaphthoxy choline iodide (Dejac Laboratories); 25 mg Blue B salt; incubate 1 hour with occasional gentle shaking at room temperature.

Esterase. 50 ml Tris-maleate buffer, 0.1 M, pH 6.4; 2 ml 1% alpha-naphthyl acetate in 50% acetone; 25 mg Blue B salt; incubate 1 hour with occasional gentle shaking at room temperature.

Alkaline phosphatase. 50 ml Tris (not Tris-maleate) buffer, 0.1 M pH 8.8; 50 mg sodium alpha-naphthyl phosphate; 50 mg Blue RR salt; 5 drops of 10% aqueous solution magnesium chloride; incubate 16 hours at room temperature or 6 hours at 37°C.

Acid phosphatase. 50 ml acetate buffer, 0.1 M, pH 5.0; 50 mg sodium alpha-naphthyl phosphate; 50 mg Red 3 GS salt; 5 drops of 10% solution of magnesium chloride; 5 drops of 10% solution manganese chloride; 1 g sodium chloride; 0.25 g polyvinylpyrollidone; incubate 24 hours at room temperature (22°C).

Cytochrome oxidase. 50 ml phosphate buffer, 0.1 M, pH 7.5; 2 ml 1% alpha naphthol in 40% ethyl alcohol; 2 ml aqueous 1% dimethylparaphenylenediamine; incubate 2 hours with occasional gentle shaking at room temperature.

Succinic dehydrogenase. 2.5 ml phosphate buffer, 0.2 M, pH 7.6; 2.5 ml 0.2 M sodium succinate; 5 ml aqueous solution of Nitro B T, 1 mg per ml; 0.25 ml phenazine methosulfate solution, 5 mg per ml; incubate 1 hour at 37°C.

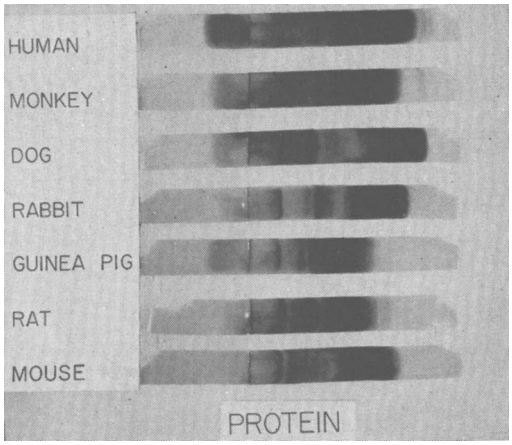

FIG. 1. Comparison of starch gels of 7 mammalian species stained for protein. (Not differentially enlarged.)

Lactic dehydrogenase. 1.0 ml of 1.0 M aqueous solution of sodium DL-lactate; 1.0 ml of 0.1 M aqueous solution of DPN (prepared from a 1.0 M solution kept at a slightly acid pH in the deep freeze); 1.0 ml of 0.1 M aqueous solution of sodium cyanide; 1.0 ml of 0.05 M aqueous solution of magnesium chloride; 2.5 ml 0.1 M phosphate buffer, pH 6.8; 2.5 ml aqueous solution Nitro B T, 1 mg per ml; distilled water to make a total of 10 ml; incubate 1 hour at 37°C.

Beta-hydroxybutyric dehydrogenase. The conditions for this enzyme were identical with those for lactic dehydrogenase, except that 1.0 ml of 1.0 M aqueous solution of DL-beta-hydroxybutyric acid was used as substrate.

Glutamic dehydrogenase. The technic was identical with that for the above 2 enzymes, except that 1.0 ml of 1.0 M aqueous sodium L-glutamate monohydrate was used as substrate.

Results. Serum protein distribution of the various species is shown in Fig. 1. In all species, reference is made to the nomenclature employed by Smithies for man(1). It may not be inferred that proteins which have similar mobilities in starch gels are identical. The protein patterns of all experimental animals were less complex than those of man. Pre-albumin was observed in only 3 species, human, monkey and mouse. Mouse and rat

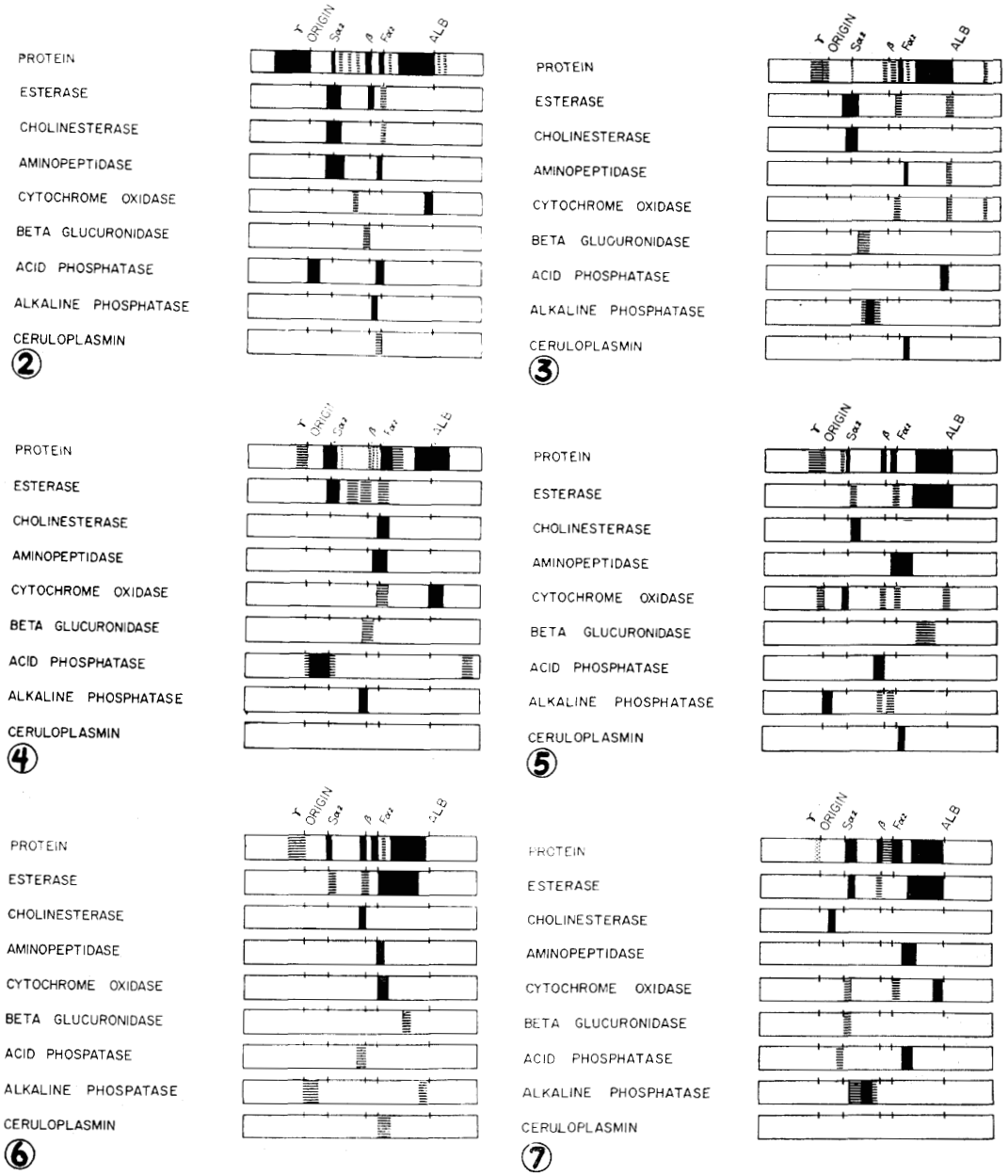

FIG. 2. Enzyme distribution in human. Dashed bands represent less activity than solid.
FIG. 3. Enzyme distribution in the monkey.
FIG. 4. Enzyme distribution in the dog.
FIG. 5. Enzyme distribution in the rabbit.
FIG. 6. Enzyme distribution in the guinea pig.
FIG. 7. Enzyme distribution in the rat.

serum samples exhibited very low gammaglobulin levels.

Fig. 2-8 illustrate occurrence and distribution of enzymes in each species. The full complement of enzymes was found in all species except the dog and the rat in which ceruloplasmin oxidase could not be demonstrated. Greatest esterase activity, as judged by width of bands and intensity of staining, was found in the mouse. Cholinesterase was observed in

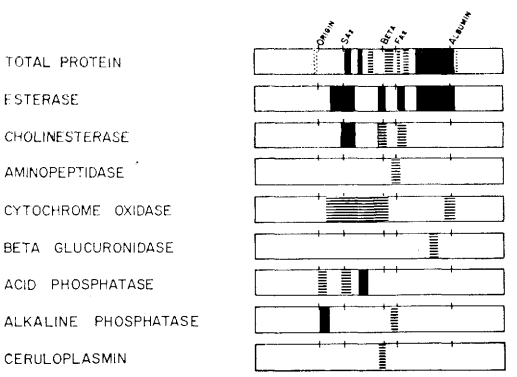

FIG. 8. Enzyme distribution in the mouse.

fractions with low mobility in all species except the dog. Cytochrome oxidase exhibited the greatest divergence with respect to number of bands, varying from one in the guinea pig to 5 in the rabbit. Beta-glucuronidase activity was found in only one band in all species. It, along with aminopeptidase and ceruloplasmin, had the most uniform migration. Although there was some variability in intensity of the various zones after both protein and enzyme stains among various members of the same species, the overall pattern for that species was constant.

Summary. The occurrence and distribution of serum enzymes and proteins in 7 mammalian species have been studied by means of starch gel electrophoresis and appropriate staining technics. Species differences were observed with respect to the presence, number, mobility, and concentration of components exhibiting various enzymatic activities. Similar observations were also made with respect to bands staining for protein.

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Comparison of "Meal Eating" and "Nibbling" on Severity of Alloxan Diabetes.* (26181)

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That rate of ingestion of diet plays a significant role in regulation of over-all body metabolism is suggested by the following data: animals fed full-spaced meals ("meal eaters") demonstrate (a) increased quantities of body fat, (b) decreased body protein, (c) altered thyroid activity and (d) changed tissue enzymatic activity, when compared to animals eating the same diet in frequent small feedings ("nibblers")(1). To determine whether manner of eating plays a role in severity of diabetes, experiments were undertaken dealing with relationship of eating habits to glycosuria and nitrogen excretion in alloxanized rats. Results indicate that rate of ingestion of diet does influence excretion of both substances in presence of experimental alloxan diabetes.

Methods. Male Holtzman rats, received when approximately 100 g in weight, were placed on a 50% carbohydrate diet *ad libitum,* and when about 130 g were given 50

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